PATENT
Attorney Docket No.: 014907-003310US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Examiner: P. Baskar
LEE et al.	Art Unit: 1645
Application No.: 09/754,947	DECLARATION OF GUNARS E. VALKIRS UNDER 37 CFR § 1.132
Filed: January 4, 2001	
For: ASSAYS FOR DETECTION OF BACILLUS ANTHRACIS	

Assistant Commissioner for Patents
Washington, D.C. 20231

I, Gunars E. Valkirs Ph.D., being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I am currently Vice President of Biosite Discovery, a subsidiary of Biosite, Inc., San Diego, CA, the assignee of U.S. Patent Application No. 09/754,947 ("the '947 application"). I am named as an inventor on the '947 application.

3. I received my Ph.D. in physics from the University of California, San Diego in 1982. I have been engaged in research and development of immunoassays and immunoassay devices since 1982.

4. I have reviewed and am familiar with the specification filed in the '947 application, the most recent Office Action mailed on June 25, 2003, and the data obtained from the Centers for Disease Control and Prevention ("CDC"), United States Department of Health and Human Services, which is attached to this declaration as Appendix A.

5. The CDC purchases two antibodies from Biosite, Inc. for the detection of *Bacillus anthracis.* These antibodies, which are referred to in the title of the CDC data, are the antibodies labeled in the present specification as "IIT005.1.C.11.1" (a recombinantly produced polyclonal) and "IIT005.1.C.11" (a recombinantly produced monoclonal). *See, e.g.*, page 51 of the '947 application. Each of these antibodies specifically bind to the *B. anthracis* surface array protein as set forth in SEQ ID NO: 1 of the '947 application, and were produced using the methods described in detail in the '947 application. As noted in the title of the CDC data, these antibodies have been used by the CDC to develop a time-resolved fluorescence assay specific for *B. anthracis.*

6. As described in the '947 application, other polyclonal and monoclonal antibodies have also been produced that specifically bind to the *B. anthracis* surface array protein as set forth in SEQ ID NO: 1 of the '947 application. *See, e.g.*, Example 5, beginning on page 46 of the specification filed in the '947 application.

7. The ability of the antibodies described in the '947 application to specifically bind *B. anthracis* was demonstrated using the Sterne strain of *B. anthracis.* *See, e.g.*, Example 6, beginning on page 48 of the specification filed in the '947 application. The Sterne strain is commonly used in order to avoid the use of dangerous

pathogenic anthrax bacteria in a laboratory environment, and is widely accepted by those of skill in the art as a surrogate for virulent *B. anthracis* strains (whether in the environment or otherwise). Indeed, to be of any use, a vaccine strain must sufficiently resemble virulent strains to provide protective immunity against those strains. Thus, the fact that the Sterne strain was developed as a vaccine strain confirms that it is considered by artisans to be a surrogate for virulent *B. anthracis*.

8. The specificifity of the antibodies described in the '947 application has been confirmed by the CDC, which determined that antibodies described in the present application recognized 36 different *B. anthracis* strains but did not bind strains from other Bacillus species. Indeed, the '947 application itself confirms that the antibodies described therein did not react with several non-*anthracis* Bacillus species. *See, e.g.*, Example 7, beginning on page 50 of the specification filed in the '947 application. Based on these data, it is my opinion that the skilled artisan would consider these results to indicate that the *B. anthracis* antibodies described in the '947 application are capable of detecting any *B. anthracis* strain and is specific for *B. anthracis*, as compared to other Bacillus species.

9. On page 5 of the Office Action mailed on June 25, 2003, the Examiner states that "the specification teaches a specific antibody that binds to SAP but fails to teach antibodies that bind to different epitopes. Additionally, it is unclear to what epitope the two antibodies bind." It is my opinion that, given the size of the surface array protein compared to the size of an average epitope recognized by an antibody (785 amino acids, compared to an average epitope size of about 15-20 amino acids), the skilled artisan would not seriously question whether two antibodies could be obtained that bind to different epitopes on the surface array protein. Moreover, the skilled artisan would also expect that a polyclonal antibody preparation, such as that described in the '947 application, would bind to multiple epitopes on the surface array protein. Finally, Example 7 beginning on page 50 of the specification explicitly teaches sandwich

immunoassays using appropriate antibody pairs. As noted above, one of these pairs (shown in Table 2) represents the antibodies purchased by the Centers for Disease Control and Prevention from Biosite, Inc. for the detection of *Bacillus anthracis*. Thus, regardless of whether the '947 application discloses explicitly which epitopes of the surface array protein any particular antibody binds to, it is my opinion that the skilled artisan would acknowledge that appropriate antibody pairs could be readily prepared and identified.

10. Based on the foregoing, it is my opinion that the skilled artisan would reasonably conclude that, by using the '947 application as a guide, and using only readily available starting materials combined with methods that were well known in the art, antibodies that specifically bind to the *B. anthracis* surface array protein as referred to in the present claims could be readily obtained. It is my further opinion that the skilled artisan could practice the claimed detection methods and provide the claimed kits using readily available starting materials, and methods that were well known in the art and the '947 application as a guide.

11. None of the references cited by the Examiner as anticipating the claims teach antibodies with the specificity expected for antibodies raised against the *B. anthracis* surface array protein (SEQ ID NO:1). In agreement with the CDC data, we have determined that the anti-*B. anthracis* surface array protein antibodies described in the '947 application and referred to in the present claims bind to vegetative *B. anthracis* cells, but do not bind to surface antigens present on *B. anthracis* spores. As described in the present specification, *e.g.*, on page 45, line 18, through page 46, line 2, the surface array protein can also be detected using the *B. anthracis* antibodies described in the '947 application in culture supernatants, such as supernatants that could be obtained by allowing *B. anthracis* to grow to the spore stage.

12. The Phillips *et al. FEMS Microbiol. Immunol.* 1988 publication cited by the Examiner discloses three antibody preparations: a monoclonal raised against intact spores (denominated "E12"), a monoclonal raised against SDS-extracted spores (denominated "A9"), and "hyperimmune mouse serum" raised against spores. The publication states that neither the E12 nor the A9 antibodies react with *B. anthracis* vegetative cells. *See, e.g.*, page 175, left column, last paragraph. In contrast, as discussed above in paragraph 11, SAP is found in vegetative cells, and the SAP-specific antibodies as recited in the claims bind to vegetative cells. Therefore, since the cited antibodies do not bind to vegetative cells, whereas the claimed antibodies do, the antibodies described in the Phillips *et al.* reference cannot describe antibodies that specifically bind to a *Bacillus anthracis* surface array protein as set forth in SEQ ID NO:1. Thus, it is my opinion that the skilled artisan would conclude that neither of the cited monoclonal antibodies is directed to the *B. anthracis* surface array protein, as required by the present claims.

13. As for the hyperimmune mouse serum disclosed in the Phillips *et al. FEMS Microbiol. Immunol.* 1988 publication, this serum was raised against whole spores (*see, e.g.*, page 171, right column, second full paragraph), and recognized both whole spores and vegetative cells (*see, e.g.*, Tables 3, 4, and 5). As discussed in paragraph 11 above, surface array protein is not available to antibodies on whole spores. Therefore whole spores should not raise antibodies that bind to surface array protein. Indeed, antibodies that specifically bind to a *Bacillus anthracis* surface array protein as set forth in SEQ ID NO:1 do not bind to whole spores (*see, e.g.*, CDC data). Therefore it is my opinion that the skilled artisan would conclude that the hyperimmune mouse serum disclosed in the publication is substantially different from the antibodies referred to in the claims, and does not specifically bind to *B. anthracis* surface array protein as required by the present claims.

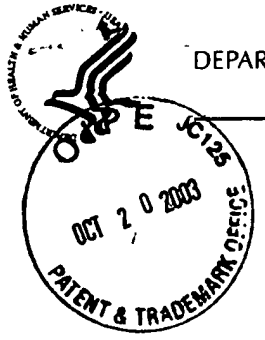
14. Similarly, the Phillips *et al. J. Appl. Bacteriol.* 1988 publication cited by the Examiner discloses hyperimmune mouse serum raised against whole formaldehyde-treated spores (*see, e.g.*, page 49, left column, first full paragraph). Formaldehyde treatment would cross-link the surface array protein within the spore and would not extract surface array protein from within whole spores. Thus, formaldehyde-treated spores would not be expected to provide surface array protein as an antigen.

15. Furthermore, only certain hyperimmune mouse serum recognized vegetative cells (*see, e.g.*, page 51, first full paragraph), and this hyperimmune mouse serum also recognized whole spores (*see, e.g.*, Table 2). Again, because the antibodies that specifically bind to the *B. anthracis* surface array protein referred to in the present claims do not bind to whole spores, it is my opinion that the skilled artisan would conclude that the hyperimmune mouse serum disclosed in this publication is substantially different from the antibodies referred to in the claims, and does not specifically bind to *B. anthracis* surface array protein as required by the present claims.

16. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements so made are punishable by fine or imprisonment or both under § 1001 of Capital Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

10/9/03
Date

Dr. Gunars E. Valkirs



DEPARTMENT OF HEALTH & HUMAN SERVICES

Office of the Secretary

Office of the General Counsel
Public Health Division
CDC/ATSDR Branch
1600 Clifton Road, N.E., M/S D53
Atlanta, Georgia 30333
(404) 639-7200

AFFIDAVIT

OLA B. CARLISLE, being first duly sworn, deposes and states as follows:

1. I am a Legal Technician in the Office of the General Counsel, Centers for Disease Control and Prevention (CDC), United States Department of Health and Human Services. I make this affidavit upon personal knowledge, and where indicated, upon the basis of information communicated to me by employees of the United States because of my official position.

2. In my position, I am a custodian of and authorized to certify the official records of CDC, an agency of the U.S. Department of Health and Human Services.

3. The attached record, imprinted with the official CDC seal, is a true copy of official records of CDC, an agency of the U.S. Department of Health and Human Services.

4. The document s referred to above are part of the official records of the United States Department of Health and Human Services.

I declare under penalty of perjury that the foregoing is to the best of my knowledge and belief true and correct.

Executed at Atlanta, Georgia.

Ola B. Di Gioia

Ola B. Di Gioia

State of Georgia)
County of DeKalb) ss

Subscribed and sworn to before me this 23rd day of September, 2003.

Ceci Velasco

Notary Public

Ceci Velasco
Notary Public, Gwinnett County, GA
My Commission Expires August 15, 2006

B. anthracis and Other Related Bacillus Strains Tested in a CDC-developed Time-Resolved Fluorescence Assay Specific for B. anthracis Cells Using *Biosite* Recombinant B. anthracis SAP (surface array protein) Fab Antibodi , IIT005.1.C.11.1 and IIT005.1.C.11

B. anthracis strains		Cells	Spores
Pasteur vaccine strain, NMRI		+	-
ASC-1	Sterne vaccine strain	+	
ASC-3	M36, Vollum derivative	+	
ASC-32	Unknown	+	
ASC-38	Human isolate	+	
ASC-45	STI, Russian vaccine	+	
ASC-58	Elephant isolate	+	
ASC-68	Ames	+	
ASC-69	New Hampshire isolate	+	
ASC-78	Q78, animal bones	+	
PB-292	Cow isolate	+	
PB-293	Cow isolate	+	
Z-1	Human isolate, Zimbabwe	+	

B. c r us strains

B. cereus negative control, NMRI			
FRI-13			
FRI-41			
FRI-42			
FRI-43			
4342		-	-

B. thuringensis strains

Alhakam			
HP571			
97-27			

CERTIFIED TO BE A TRUE COPY OF THE ORIGINAL

Ola B. DiBioia

Certifying Official

9-23-03

Date

B. anthracis strains		Cells	Spores
AO34	B. anthracis	+	-
AO39	"	+	
AO62	"	+	
AO102	"	+	
AO149	"	+	
AO158	"	+	
AO174	"	+	
AO188	"	+	
AO193	"	+	
AO248	"	+	
AO256	"	+	
AO264	"	+	
AO267	"	+	
AO297	"	+	
AO328	"	+	
AO367	"	+	
AO379	"	+	
AO419	"	+	
AO442	"	+	
AO463	"	+	
AO465	"	+	
AO489	"	+	
AO 462	Ames strain	+	
AO488	Vollum	(+) weakly reactive	